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"FORMULATION AND EVALUATION OF BIOINSPIRED NANOMEDICINE"

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INDEX

SR. NO.	*CONTENT*	*PAGE NO.*
01	*ABSTRACT OF PROPOSED WORK PLAN*	*1*
02	*INTRODUCTION*	*2*
03	*REVIEW OF LITERATURE*	*5*
04	*OBJECTIVES OF RESEARCH*	*9*
05	*MATERIALS AND METHODOLOGY TO BE ADOPTED*	*10*
06	*IMPORTANCE OF STUDY*	*11*
07	*PROPOSED WORK PLAN*	*13*
08	*REFERENCES*	*14*

1. Abstract of proposed work plan/ problem:

In the recent years, noble nanoparticles have attracted and emerged in the field of biology, medicine and electronics due to their incredible applications. There were several methods have been used for synthesis of nanoparticles such as toxic chemicals and high energy physical procedures. To overcome these, bioinspired approach has been used for the synthesis of various metal nanoparticles. The bioinspired nanocarriers are capable of carrying biologically active molecules to the target sites. This bioinspired nanosystem constitutes of lipids, polymers and biomaterials which utilizes various responsive sensors for targeted drug delivery system Moreover, the biological approach, in particular the usage of natural organisms has offered a reliable, simple, nontoxic and environmental friendly method. In biological method nanoparticles synthesis was carried out without addition of any reducing agent and the stabilizer which are replaced by molecules produced by living organism i. e. bacteria fungi, yeast, algae, and plant etc. therefore these living organism are the nanofactories for production of nanoparticles synthesis using natural organism has become a major research area in the field of nanotechnology. Hence, the current research is focused on the formulation and evaluation of bioinspired nanomedicine.

2. Introduction:

In recent years the topic of nanoparticles has received particular interest in a wide range of fields. The term "*nano*" comes from the Greek word "*nanos*" meaning dwarf and denotes a measurement on the scale of one-billionth of a metre in size. Nanoparticles are defined as particulate dispersions of solid particles with at least one dimension at a size range of 10-1000 nm. The most important feature of nanoparticles is their surface area to volume aspect ratio, allowing them to interact with other particles easier.

They are more reactive than the larger particles due to their greater surface area per weight. Nanoparticles are more stable for long period.Nanoparticles can be prepared by physical, chemical, biological, and hybrid methods. Although physical, chemical methods and green methods are more popular in the synthesis of nanoparticles. The use of toxic chemicals greatly limits their biomedical applications, in particular in clinical fields. Therefore, development of reliable, nontoxic, and eco-friendly methods for synthesis of nanoparticles is of utmost importance to expand their biomedical applications.

One of the options to achieve Nanoparticle technologies used for human administration must be designed to interact with a living host environment. The idea about bioinspired smart drug delivery carriers includes the development of biocompatible nanomaterials which can be further loaded with the drug for specific targeted drug delivery applications. Bio-inspired nanoparticles (NPs) held edges over conventionally synthesized nanoparticles due to their low cost, easy synthesis and low toxicity.

Bio-smart nanosystems are used for several applications in the delivery of drugs and pharmaceuticals for their therapeutic applications like biological markers, diagnostic purposes such as imaging applications and also for gene therapy. Thus, the bioinspired nanocarriers are capable of carrying biologically active molecules to the target sites. This bioinspired nanosystem constitutes of lipids, polymers and biomaterials which utilizes various responsive sensors for targeted drug delivery systems. However, external conditions such as heat, light, magnetic or electric field and ultrasounds, along with temperature, altered pH and ionic strength can affect the bioinspired smart nanosystem for drug delivery. Nanotechnology is the new succeeding tool for medical and biological field develops by nanotechnology. It combines biological principles with physical and chemical procedure to generate nano sized particles with specific function.

Last few years nanoparticles produced by physical and chemical method for the production of large quantities of nanoparticles which are complicated, outdated, expansive, and produce hazardous toxic wastage that are harmful to environment as well as human health also.To solving this problem and large quantity nanoparticles production the new method arises called as biological method. This is proper alternative to physical and chemical method because it is nontoxic, eco-friendly, recent, modern and safe. In biological method nanoparticles synthesis was carried out without addition of any reducing agent and the stabilizer which are replaced by molecules produced by living organism i. e. bacteria fungi, yeast, algae, plant etc. therefore these living organism are the nanofactories for production of nanoparticles.

Bacteria, fungi, yeast, algae, actinomycetes synthesis silver (Ag), gold (Au), cadmium sulphate (Cds), zinc sulphate (Zns), platinum (Pt), and palladium (Pd) nanoparticles. Out of microbes fungi are most effective good candidate in synthesis of metal Nanoparticles and large scale production, Because fungi secrete large amount of proteins and enzymes for reducing the metal ion and increasing productivity , large amount of biomass production and fungi have very high wall binding capacity. Nanoparticles synthesis from fungi known as Myconanotechnology which has great demand .Due to their monodispersity today fungi are bio nanofactories if various metal nanoparticles like silver(Ag), gold(Au), platinum(Pt), Cds. Nanoparticles production source is intracellular or extracellular which depend on the reduction enzyme present in it.

Nanoparticles have multifunctional used in various branches. Silver, gold nanoparticles have great antimicrobial property, which are used in various medicines for detection of diseases. Nanoparticles also used in fabric, in manufacturing materials, electronic devices, solving environmental problem etc. therefore biological synthesized Nanoparticles have tremendous amount of application than the chemically synthesized nanoparticles. Thus the use of microorganism to synthesized functional nanoparticles has been of great interest. Micro-organism can change the oxidation state of metals.

Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the element metal through enzymes generated by the cell activities. It can be classified into intracellular and extracellular synthesis according to the location where nanoparticles are formed. The intracellular method consists of transporting ions into the microbial cell to form nanoparticles in the presence of enzymes. The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and

reducing ions in the presence of enzymes. The biosynthesized nanoparticles have been used in a variety of applications including drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science, and magnetic resonance imaging (MRI).

Traditionally UV irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques have been used successfully to produce various nanoparticles such as gold, silver, platinum and palladium. However, considering the fast growth in the usage of nanomaterials in diverse fields, there is an urgent need to develop clean, nontoxic, simple and eco-friendly procedures for their synthesis.

Synthesis of noble metal nanoparticles, in particular silver nanoparticles (AgNPs) synthesis using natural organism has become a major research area in the field of nanotechnology. This may due to their simplicity of procedures, stability of nanoparticles, and their potential applications in chemical sensing, biological imaging, antimicrobial, gene silencing, drug delivery. Recently, several studies have reported natural polymers such as chitosan, starch and tannic acid as reducing agents for the synthesis of silver and gold nanoparticles. A vast array of biological resources including plants, algae, fungi, yeast, bacteria, and viruses has been studied so far for the intra and extracellular synthesis of silver, gold,

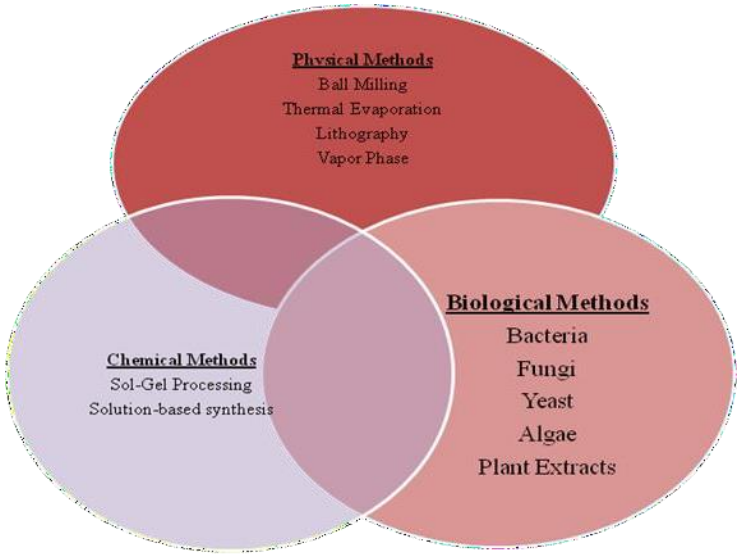

Fig. 2: Methods of Nanoparticle Synthesis

platinum and titanium nanoparticles in different sizes and shapes were available. Therefore an environment and eco friendly approach for synthesis of nanoparticles using microorganisms and

different plants commonly referred to as Bioinspired approach is the most researched area for synthesis of nanoparticles holds immense potential.

Infectious diseases can be caused by many pathogens, including bacteria, viruses, fungi, and parasites that may cause illness and disease. For humans, transmission of pathogens may occur in a variety of ways: spread from person-to-person by direct contact, water or food borne illness or aerosolization of infected particles in the environment and through insects (mosquitoes) and ticks.

There are still great challenges for the effective treatment of infectious diseases, although considerable achievement has been made by using antiviral and antimicrobial agents varying from small-molecule drugs, peptides/proteins, to nucleic acids. The nanomedicine approach is emerging as a new strategy capable of overcoming disadvantages of molecular therapeutics and amplifying their anti-infective activities, by localized delivery to infection sites, reducing off-target effects, and/or attenuating resistance development. Nanotechnology, in combination with bioinspired approaches, affords additional functions to nanoparticles derived from synthetic materials. Herein, we aim to provide a state-of-the-art review on recent progress in bioinspired and bioengineered nanotherapies for the treatment of infectious diseases. The significance of these materials is vast, ranging from applications in drug delivery and tissue engineering to sensors and diagnosis devices. Thus, innovation through bio-inspired nanomaterials has allowed great progress in biomedical applications with bio-mimetic capabilities, such as robotic nanodevices, organs-onchips and tissue engineering.

3. Review of Literature and Development in the subject (Previous work done in the relevant area)

1. **Suntia Patil et. al.(2020)** formulated and evaluated biological silver nanoparticle gel (BSNG) against common wound infection causing bacteria. The antibacterial activity of formulated gel was compared with the marketed antibiotic formulations (MAF) containing silver sulphadiazine (1 % w/w), metronidazole (1 % w/w) and chlorhexidine gluconate (0.20 % w/w). The antibacterial activity of biological was analysed by well diffusion method.
2. **Prathima Srinivas et. al.(2020)** proposed for the Antifungal activity of biosynthesized silver nanoparticles from Candid albicans on the strain lacking the CNP41 gene, proposed the potential usage of wild-type C. albicans strain C86 to biosynthesize silver nanoparticles by microwave assisted technique. Visual colour change and UV spectrophotometer were used for primary detection of silver nanoparticles. Additionally, the FTIR peaks confirm the particles' formation and surface characterization techniques such as FESEM and EDX suggests that the silver nanoparticles were sized in the range of 30–70 nm combinational effect was studied by incorporating antifungal drug fluconazole. Both individual and enhanced combinational antifungal effects of silver nanoparticles and fluconazole were observed on genetically modified C403 strain with 40% increase in fold area compared to wild-type C86 strain.
3. **Bhartidasan R. et. al*.(2016)*** seventy one endophytic fungi isolated from Avicennia marina, Suaedamonica and Rhizophoramucronata plant leaf. Among 34 genera and 71 species was identified by Lactophenol cotton blue mounting techniques. To induce the biosynthesis of silver nanoparticles (AgNO3) using Aspergillus conicus, Penicillium janthinellum and Phomosis sp. and evaluate their antimicrobial potential. The characterization of silver nano particles on these fungi by FT-IR, UV, and Fre-SEM analysis to confirm the reduction and it is believed that protein might have played an important role in the stabilization of silver nanoparticles. The synthesized AgNO3 were found to be extracellular poly dispersed spherical or hexagonal particles ranging from 6-12mm, 8-14mm 10-16mm in size. Antibacterial activity was performed using a Agar well diffusion method against Bacillus subtillus, E-coli, Enterobacteraurogens, Enterococcus, Klebseilla Pneumonia, Micrococus lutes, Salmonella typhi, Staphylococus aureus Streptococcus sp. Vibrio cholera. AgNO3 can be myco synthesized extracelluraly using A.Conicus, Penicillium janthinellum and Phomosis sp. As the fungal system.

4. **Vasudeva Reddy Netala et. al. (2016)** isolated an endophytic fungal strain from the leaves of Gymnema sylvestre was identified as Pestalotiopsis microspora VJ1/VS1 based on nucleotide sequencing of internal transcribed spacer region (ITS 1-5.8S-ITS 2) of 18S rRNAgenean efficient and ecofriendly approach has been reported for the synthesis of silver nanoparticles (AgNPs) using aqueous culture filtrate of P. microspora. Ultraviolet- visible analysis confirmed the synthesis of AgNPs by showing characteristic absorption peak at 435 nm. Fourier transform infrared spectroscopy analysis revealed the presence of phenolic compounds and proteins in the fungal filtrate, which are plausibly involved in the biosynthesis and capping of AgNPs. Transmission electron microscopy (TEM) showed that the AgNPs were spherical in shape of 2–10 nm in size. Selected area electron diffraction and X-ray diffraction studies determined the crystalline nature of AgNPs with face-centered cubic (FCC) lattice phase. Dynamic light scattering analysis showed that the biosynthesized AgNPs possess high negative zeta potential value of -35.7 mV. Biosynthesized AgNPs were proved to be potential antioxidants by showing effective radical scavenging activity against 2,2′-diphenyl-1-picrylhydrazyl and H2O2 radicals with IC50 values of 76.95±2.96 and 94.95±2.18 μg/mL, respectively.
5. **Iravani S et. Al.** (2016) the ability of C. Vulgaris and Oscillatoriawillei to synthesize silver NPs [64]. C. Vulgaris biosynthesized silver nanoparticles in a rod-like shape with a mean length of 44 nm and width of 16 – 24 nm, while Oscillatoriawillei biosynthesized silver NPs with a diameter range of 100 – 200 nm.
6. **Ashish Garg et. al. (2018)** Heparin modified-cellulose acetate copolymer was manufactured by precipitation method and was substantiated by Fourier-transform infrared spectroscopy and nuclear magnetic resonance spectroscopy. HEC NPs with UA was formulated by employing HEC copolymer and later competed with UA-loaded cellulose acetate phthalate (CAP) NPs. NPs were exemplified by zeta potential, differential scanning calorimetry, particle size, atomic force microscopy, in vitro release, entrapment efficiency, X-ray diffraction , and polydispersity index. Studies revealed that HEC NPs have a slower release (96.21% in 32 h) when contrasted with CAP NPs (97.36% in 8 h). In cytotoxicity analysis of A549, UA-loaded HEC NPs illustrated an immense cytotoxic potential. In addition, HEC NPs were found to be more hemocompatable in comparison to CAP NPs and plain UA.
7. **Yue Zhang et. al. (2018)** reported an erythrocyte membrane-coated nanogel (RBC-nanogel) system with combinatorial antivirulence and responsive antibiotic delivery for the treatment of

methicillin-resistant Staphylococcus aureus (MRSA) infection. RBC membrane was coated onto the nanogel via a membrane vesicle templated in situ gelation process, whereas the redox-responsiveness was achieved by using a disulfide bond-based crosslinker. We demonstrated that the RBC-nanogels effectively neutralized MRSA-associated toxins in extracellular environment and the toxin neutralization in turn promoted bacterial uptake by macrophages. In intracellular reducing environment, the RBC-nanogels showed an accelerated drug release profile, which resulted in more effective bacterial inhibition. When added to the macrophages infected with intracellular MRSA bacteria, the RBC-nanogels significantly inhibited bacterial growth compared to free antibiotics and non-responsive nanogel counterparts. These results indicate the great potential of the RBC-nanogel system as a new and effective antimicrobial agent against MRSA infection.

4. Objectives of Research/ Proposed Hypothesis:

Aim: Formulation and evaluation of bioinspired nanomedicine.

Objectives of the Research Work:

- To formulation and evaluation of bioinspired nanomedicine.
- To improve the development of biocompatible nanomaterials which can be further loaded with the drug for specific targeted drug delivery applications by formulating bioinspired nanomaterials.
- To produce a reliable, simple, nontoxic and environmental friendly method.
- To prepare and evaluate Bioinspired nanoparticles.
- To minimize the toxicity and increase the stability of drug by encapsulating in bioinspired nanomedicine.
- To optimize the developed formulation.
- Physicochemical, in-vitro and in-vivo evaluation of optimized formulation.
- To study stability of optimized formulation.

5. Materials and Methodology to be adopted:

1. **Literature review**
2. **Selection and Collection of Drug and Excipients**

   Drug:-Antifungal or antibacterial etc**.**

   Excipients: To formulate the bioinspired nanoparticles, one of the following biological species can be used. Haluronic acid or heparin or algae or fungi or yeast etc.

3. **Experimental work**

A. Preformulation Study of drug

1. Spectroscopic studies
2. Solubility studies
3. Crystallinity studies

B. Compatibility studies of drug and excipient

4. **Formulation of bioinspired nanomedicine**

- **Characterization of drug-loaded bioinspired nanomedicine**

  - Yield and drug content
  - Particle size and zeta potential
  - Encapsulation efficiency
  - Surface morphology by Transmission electron microscopy (TEM)
  - Fourier transform infrared spectroscopy
  - PXRD study
  - Differential scanning colorimetry
  - In vitro drug release

5. **In vitro study**
6. **In vivo animal study**
7. **Stability studies**

6. Importance of study/ Society application:

- **International status:** There are still great challenges for the effective treatment of infectious diseases, although considerable achievement has been made by using antiviral and antimicrobial agents varying from small-molecule drugs, peptides/ proteins, to nucleic acids. The nanomedicine approach is emerging as a new strategy capable of overcoming disadvantages of molecular therapeutics and amplifying their anti-infective activities, by localized delivery to infection sites, reducing off-target effects, and/or attenuating resistance development. Nanotechnology, in combination with bioinspired approaches, affords additional functions to nanoparticles derived from synthetic materials. Herein, we aim to provide a state-of-the-art review on recent progress in bioinspired and bioengineered nanotherapies for the treatment of infectious diseases. Different bioinspired nanoparticles, derived from biological cells, are developed with the applications in diagnosis, imaging, therapy are introduced, either by their intrinsic activity or by loading and site-specifically delivering various molecular drugs.
- **National status:** India, being a country of extreme geo-climatic diversity, faces a constant threat of emerging and re-emerging viral infections of public health importance. There is a need for strengthening disease surveillance in the country focusing on the epidemiology and disease burden. There is also a pressing need to gain detailed insights into disease biomics, including vector biology and environmental factors influencing the diseases. It is also important to strengthen the emergency prepared for these diseases and response by focusing on 'one health' approach. Bioinspired nanoparticles will be the better approach to efficiently overcome disease burden. Due to their biocomposition of microorganisms which have the innate potential for the synthesis of nanoparticles and they could be regarded as potential biofactories for nanoparticles synthesis.

►Significance of the study:

Nanoparticles are the fundamental building blocks for preparing many nanostructured materials and devices. Therefore, there is an enormous interest in developing safe, cost effective and ecofriendly techniques for synthesis of nanoparticles. Increasing awareness towards green chemistry and biological processes has led to a desire to develop an environment-friendly approach for the synthesis of non-toxic nanoparticles. Unlike other processes such as physical and chemical methods, which involve hazardous chemicals. In particular, Bio-inspired nanomaterials are emerging as a promising field of research with a high potential for developing unprecedented approaches for managing human diseases such as an antibacterial, antifungal activity etc. The significance of these materials is vast, ranging from applications in drug delivery and tissue engineering to sensors and diagnosis devices. They show great promise in terms of biomedical applications *viz,* wound healing properties, antimicrobial properties, anti-inflammatory properties, etc. Current research is revealed that the disease causing microbes have become resistant to drug therapy. The bioispired nanocarriers provides an excellent platform for reasons of being an easily modifiable, cost-effective, and safer alternative to the other microbiome derived counterparts. So this environmental friendly method was analyzed to be faster when compared to other chemical methods and was evaluated as the very good choice of antimicrobial agent. This has urged upon the need to develop safe, reliable and ecofriendly approaches for large scale production of silver nanoparticles. Thus, innovation of bio-inspired nanomaterials is needed to overcome large variety of infectious diseases.

7. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved:

<table>
<tr><th>Sr. No.</th><th>Expected target to be achieved</th><th>1-6 months</th><th>7-10 months</th><th>11-22 months</th><th>22-27 months</th><th>28-33 months</th><th>34-36 months</th></tr>
<tr><td>01</td><td>Literature survey</td><td>Literature survey</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>02</td><td>Selection and collection of drug and excipients</td><td colspan="2">Selection and collection of drug and excipients</td><td></td><td></td><td></td><td></td></tr>
<tr><td>03</td><td>Formulation of bio-inspired nanomedicine</td><td colspan="3">Formulation of bio-inspired nanomedicine</td><td></td><td></td><td></td></tr>
<tr><td>04</td><td>Evaluation of bio-inspired nanomedicine</td><td colspan="4">Evaluation of bio-inspired nanomedicine</td><td></td><td></td></tr>
<tr><td>05</td><td>In vitro and in vivo study</td><td colspan="5">In vitro and in vivo study</td><td></td></tr>
<tr><td>06</td><td>Statistical analysis, data interpretation and conclusions</td><td colspan="6">Statistical analysis, data interpretation and conclusions</td></tr>
<tr><td>07</td><td>Publishing research and submitting data</td><td colspan="6">Publishing research and submitting data</td></tr>
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